活用 TAIR 於植物研究:阿拉伯芥與作物的實用工具 Using TAIR for Plant Research: Practical Tools for Arabidopsis and Crops



The Arabidopsis Information Resource



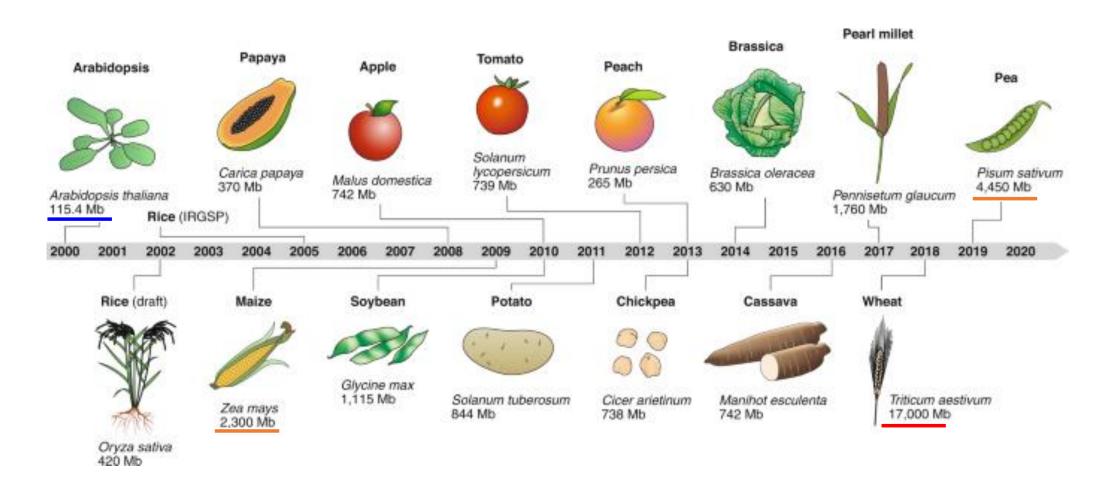
Ho-Ming Chen (陳荷明)

- Associate fellow, Agricultural Biotechnology Research Center (ABRC), Academia Sinica
- 25 years of experience in bioinformatics
 - Data: Microarray, Small RNA, RNA degradome, RNA-Seq, Ribo-seq
 - Species: Arabidopsis (model diploid), banana (crop; triploid), rice, tomato etc

Why TAIR database?

- You can do fewer experiments, take fewer wrong turns, and graduate sooner
- You can do biology research from a different perspective, surpass your supervisor, and become a next-generation plant researcher.

Timeline of plant genome release

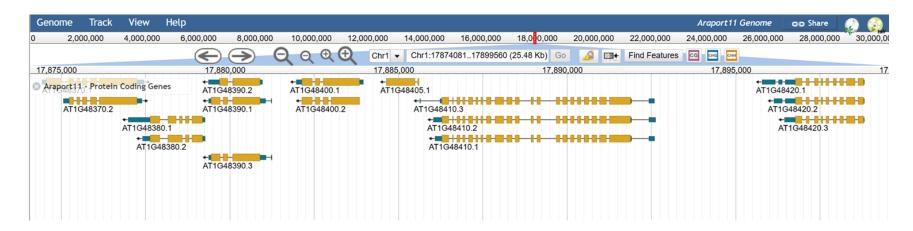


https://www.nature.com/articles/s41588-021-00866-3

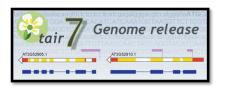
Arabidopsis genome annotation

- High quality sequence with few gaps
- High quality of genome annotation (frequent update)

Genome annotation: The process of identifying the locations of genes and all of the coding regions in a genome and determining what those genes do.











TAIR10

Araport11

TAIR annotations among versions

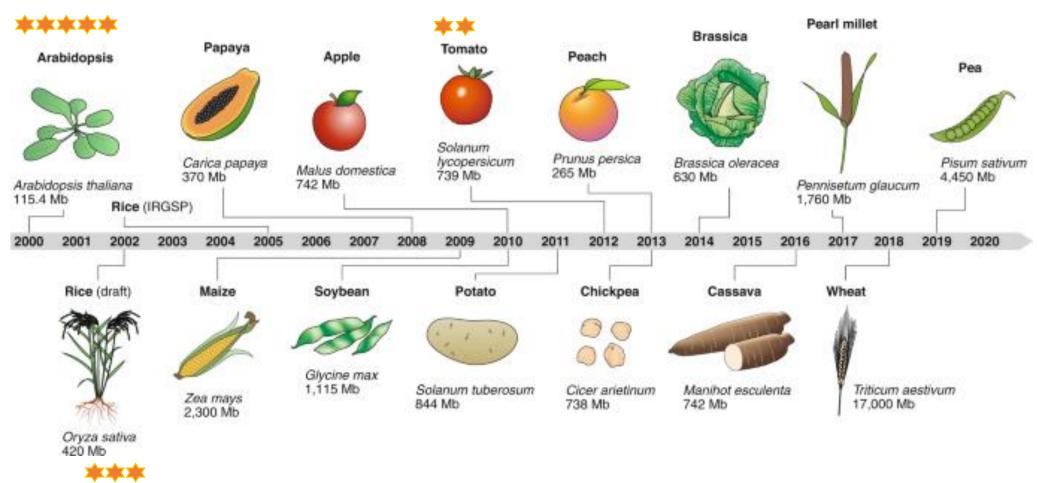
All Genome Versions Statistics							
	Protein Coding Genes	Transposons and pseudogenes	Alternatively spliced genes	Gene density (Kb/gene)	Avg. exons per gene	Avg. exon length	Avg. intron length
TAIR10 (11/10)	27,411	4,827	5,885	4.35	5.89	296	165
TAIR9 (6/09)	27,379	4,827	4,626	4.35	5.67	304	165
TAIR8 (4/08)	27,235	4,759	4,330	4.37	5.62	306	165
TAIR7 (4/07)	26,819	3,889	3,866	4.44	5.79	268	165
TAIR6 (11/05)	26,541	3,818	3,159	4.48	5.64	269	164
TIGR5 (1/04)	26,207	3,786	2,330	4.54	5.42	276	164
TIGR4 (4/03)	27,170	2,218	1,267	4.38	5.31	279	166
TIGR3 (8/02)	27,117	1,967	162	4.32	5.24	266	166
TIGR2 (1/02)	26,156	1,305	28	4.48	5.25	265	167
TIGR1 (8/01)	25,554	1,274	0	4.55	5.23	256	168
Nature (12/00)	25,498	NA	NA	4.50	5.20	250	168

Araport11	Change
27 655 ^a	+239
48 359	+12 973
10 696	+4892
-	+1158
-	+21 298
84	+26
	48 359 10 696 -

Cheng et al., (2017) Plant Journal

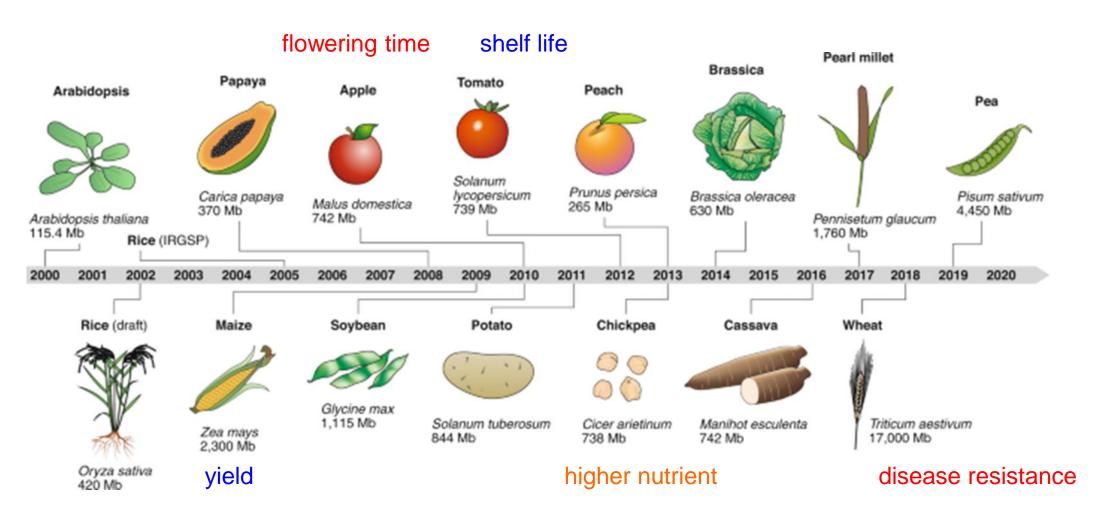
Quality of plant genomes

Genome assemblies and annotations



https://www.nature.com/articles/s41588-021-00866-3

Translational agricultural research



Finding target genes in crops

Early flowering

ChatGPT ~

I would like to use genome editing to create an early flowering orange line. Please suggest two targets to edit.

Thought for 12s

Nice — good goal. Below are **two high-confidence gene targets** you can consider editing in orange (Citrus) to promote earlier flowering, with concise rationale, likely phenotypes, and key caveats. I'll keep this at a conceptual, non-actionable level (no editing protocols or sequence/guide design).

2) A citrus TFL1 / CEN homolog (e.g., *CsTFL1* / TFL1-like gene) — *loss-of-function* / *repression*

Why: TFL1 (and CEN/TFL1-like genes) antagonize FT and maintain meristem indeterminacy; loss of TFL1 function typically releases meristems to flower earlier. TFL1 homologs in woody perennials (including citrus) have been identified and linked to flowering control.

Expected outcome: earlier and/or more frequent flowering due to reduced repression of floral meristem identity. Combining reduced TFL1 activity with moderate FT activation can be synergistic.

Oxford Academic

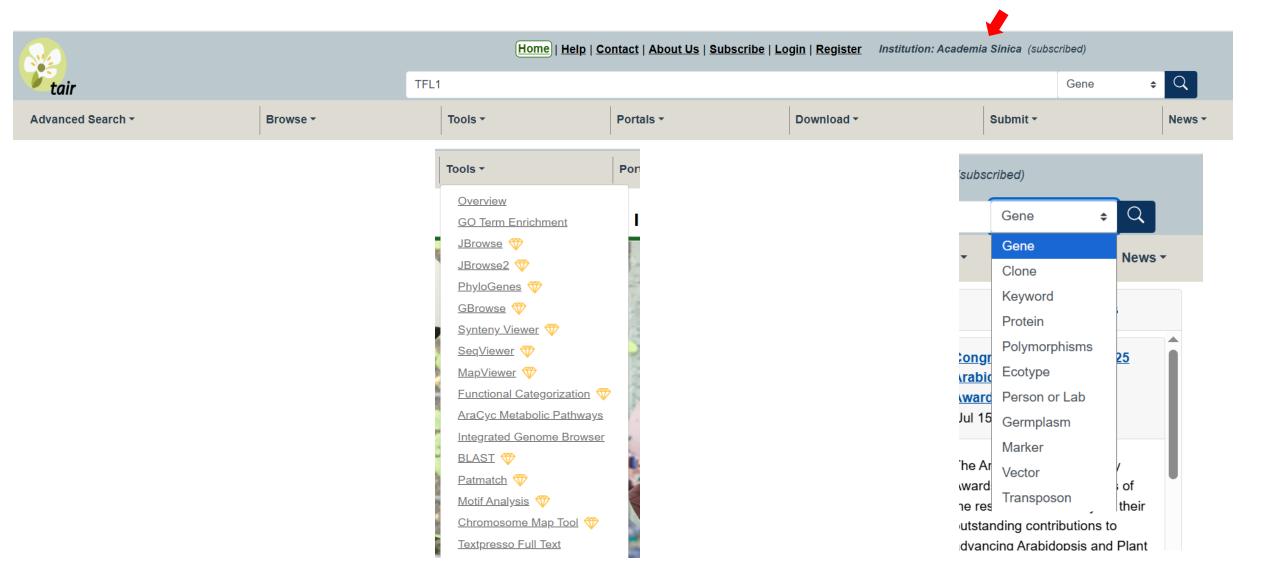
Major trade-offs/risks: loss of meristem indeterminacy can change tree form, branching pattern, and possibly long-term productivity. Complete loss-of-function can be more disruptive than controlled down-regulation.

ChatGPT can make mistakes. Check important info.

Read review papers and check TAIR database

TAIR homepage

subscribed



TAIR subscription

Sinica has institutional subscription (unlimited access)



Purchase usage units for individual academic or non-profit use 1

Subscription Benefits

- · Unlimited access to TAIR for institutions
- Up-to-date, manually curated data from the literature
- · Custom datasets on request
- · Downloadable, current genome-wide datasets

Choose License Type





Institutional Academic

Individual Academic

Recommended



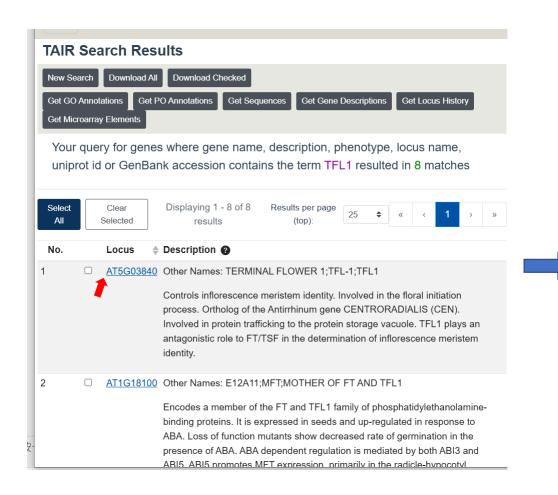
Commercial

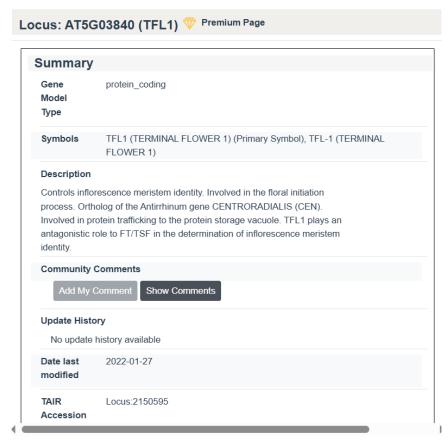
Individual Access Options

	Complimentary	Regular user	Frequent user	
Usage units	50	300	900	
Annual cost, per purchase	No purchase necessary	\$200 for first, \$500 for subsequent	\$1125	
Visit frequency	A few times a year	Once a week	Every day	
Average # of premium data detail pages visited (1 unit each/24 hr period)	About 20 a year	20 to 200 a year	300 to 400 a year	
Average # of premium downloads (25 units each/24 hr period)	1 a year	2 to 4 a year	6 to 8 a year	
Tool use (e.g., BLAST, JBrowse) (1 unit each/24 hr period)	Five or so times	20 to 50	100 to 200	

NEXT

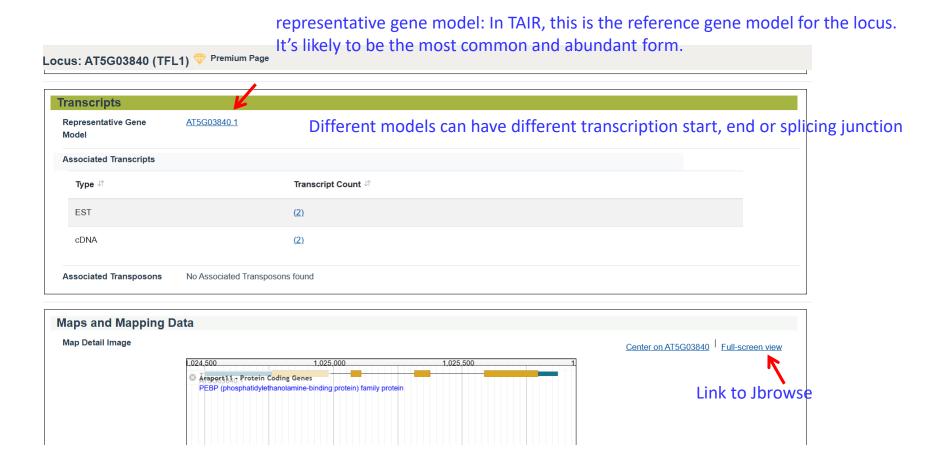
Search results and locus page





Summary

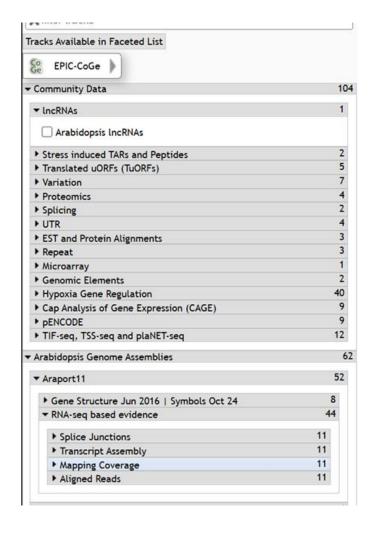
Transcripts

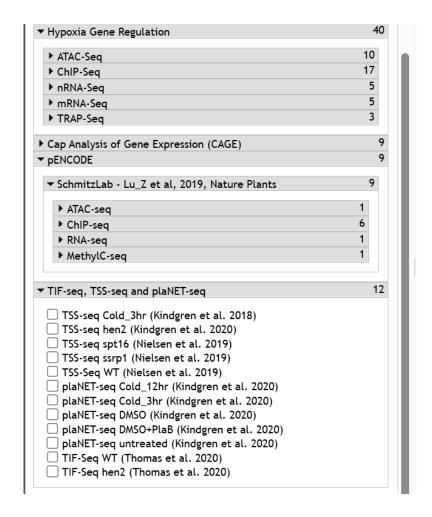


yellow box: coding exon; blue box: untranslated region (5' or 3' UTR); thin line: intron

Genome browser

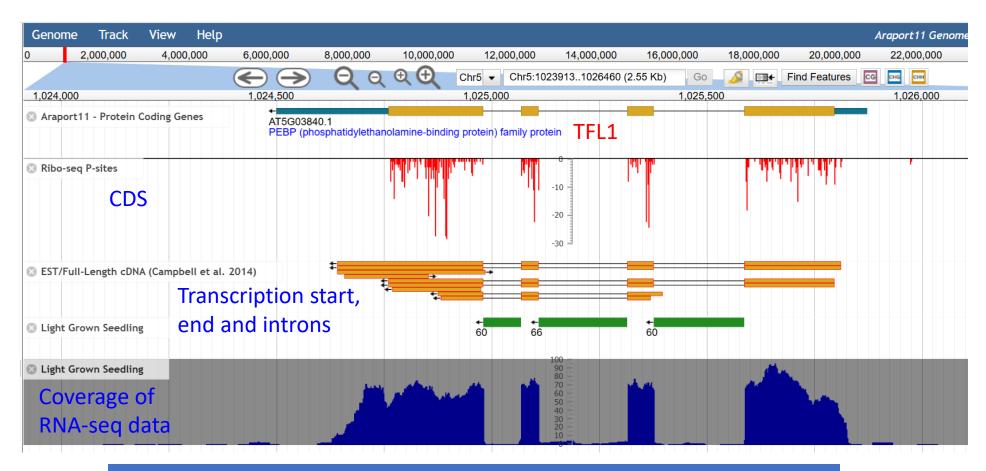
An important tool for users to visualize and browse entire genomes with annotated data or omic data, including gene structure, sequence variation, mutations, RNA-seq data, Ribo-seq data and peptide data etc.





Genome browser: Jbrowse

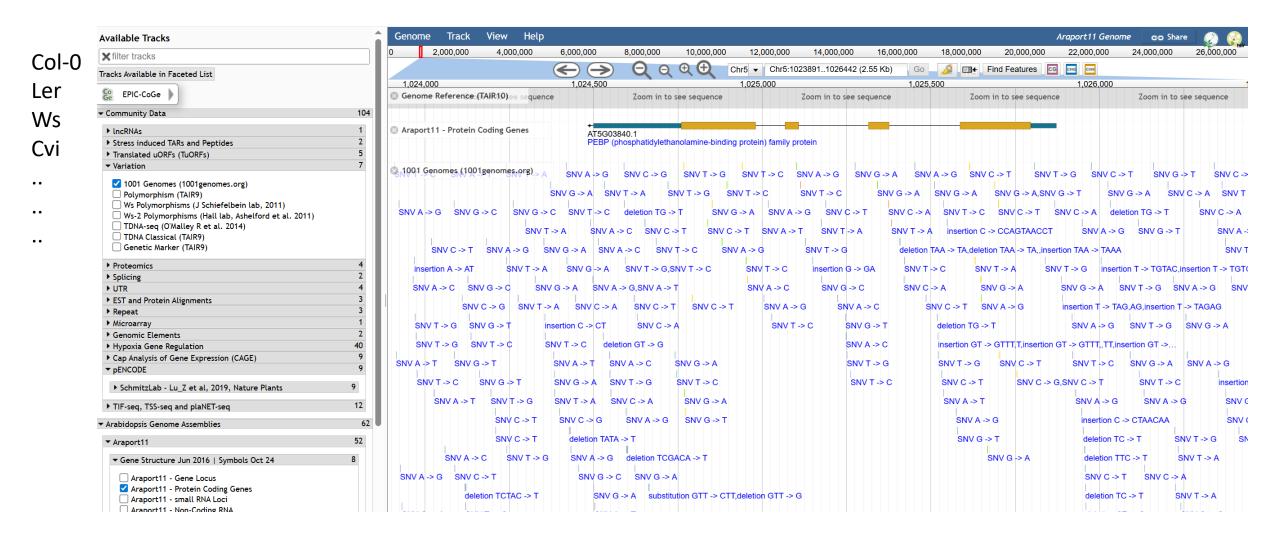
Check the accuracy of gene model (transcription start, end, introns, and CDS)



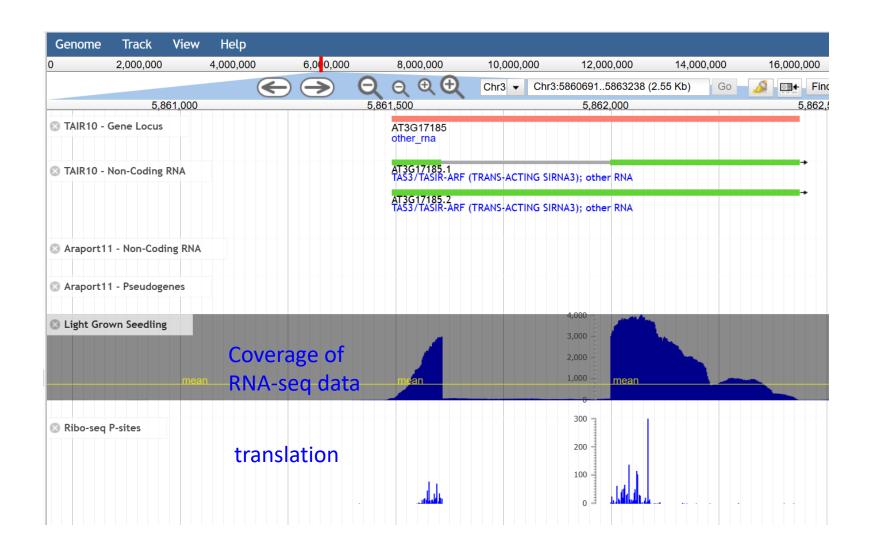
Do you agree with this model? If not, what changes will you make?

Genome browser: sequence variation among ecotypes

reference genome \neq your genome



Genome browser: hidden RNA or open reading frame



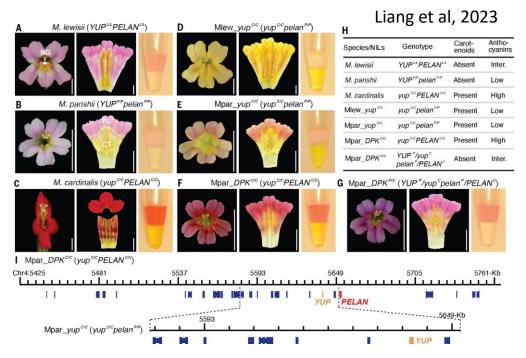
Genome browser: non-coding RNA in monkey flower

"Unusual suspects in flower color evolution"

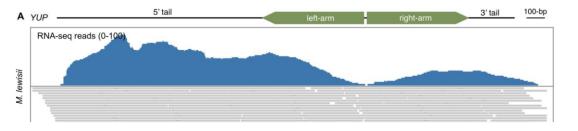


Science 2023

YUP: yellow carotenoid pigments in the petal upper epidermis



- 8 protein coding gene (RNAi no phenotype)
- 1 non-coding gene (small RNA producing locus)



Exercise I

TGA1 (AT5G65210)

- 1. number of isoforms? (the differences)
- 2. the representative form in Araport11? Do you agree (check JBrwose)? If not, your answer?
- 3. Are there any upstream open reading frames?

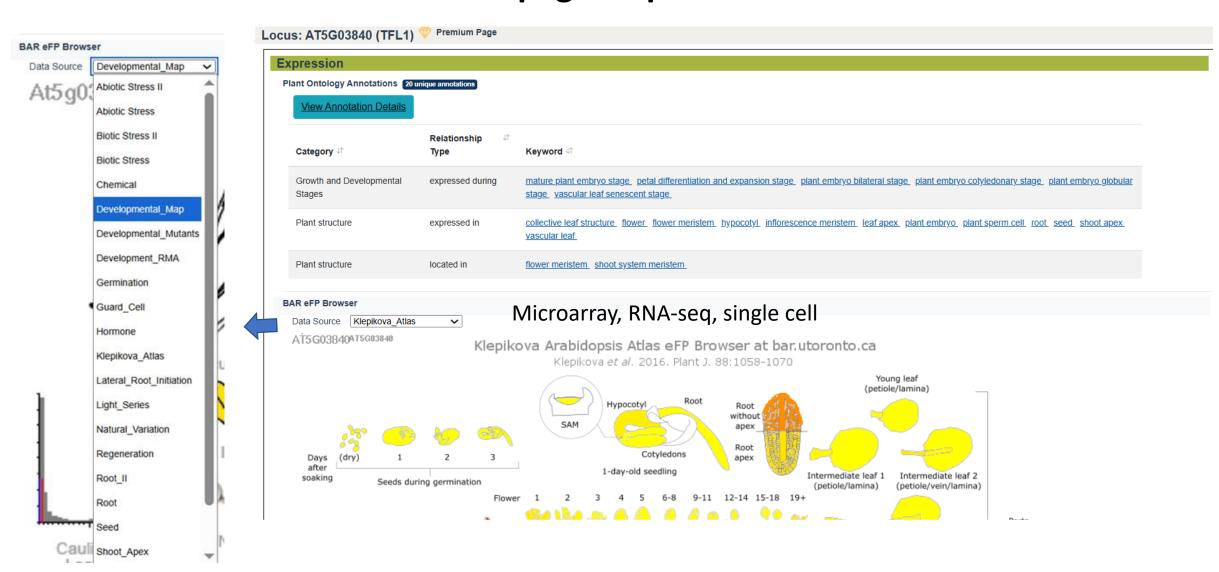


Locus page: DNA and protein sequences

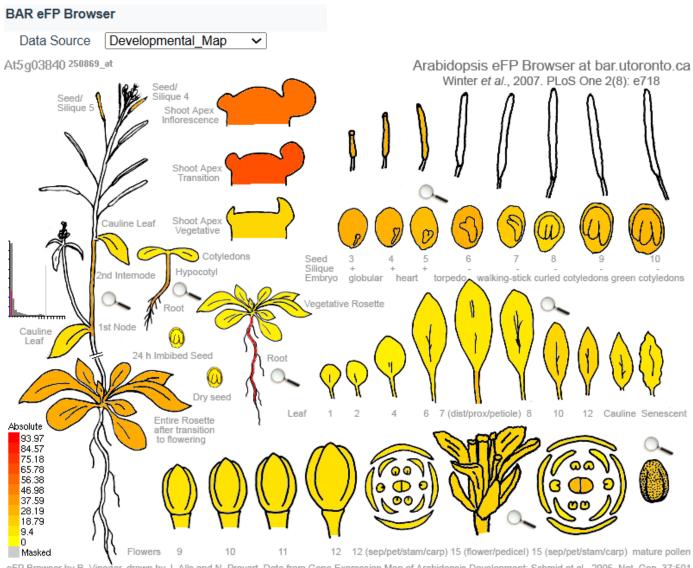
			Intron 5'UTR CDS 3' UTR	5'UTR CDS 3' UTR	CDS	Protein sequence of the representative form
Se	equences					
S	Sequence		full length genomic	full length cDNA full le	ength CDS	protein
Pi	rotein Data					
Р	rotein Data					
	Name ↓↑	Length ↓↑	Molecular Weight ↓↑	Isoelectric P	oint √	INTERPRO domains ↓↑
	AT5G03840.1	177	20158.1	10	.29	PEBP_euk:IPR035810

- Some gene models don't have 5' UTR or 3' UTR
- Some gene models have incorrect 5' UTR or 3 'UTR
- If 5' UTR or 3' UTR is important to you, compare the annotation with ESTs/full length cDNA or RNA-Seq data in JBrowse

Locus page: Expression



Development map expression



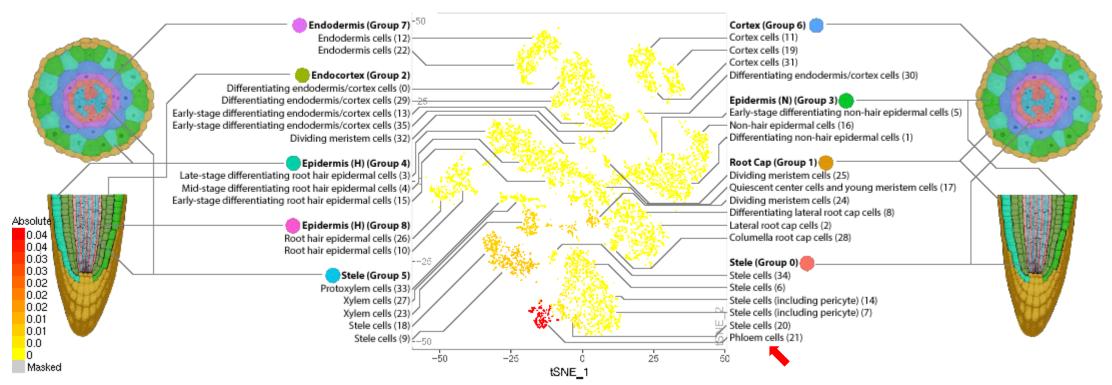
eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

Single cell expression

BAR eFP Browser					
Data Source [Single_Cell	~			
AT5G03840AT5G0	Root 8				

scRNA-Seq at bar.utoronto.ca

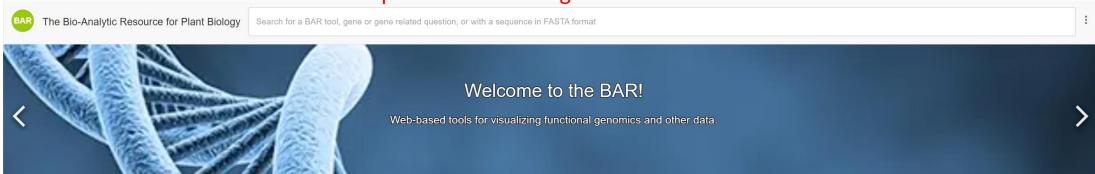
Ryu et al., Plant Physiology, DOI: 10.1104/pp.18.01482



BAR: The Bio-Analytic Resource for plant biology

https://bar.utoronto.ca/#OtherGenomicTools

Expression data and genomic tools





Gene Expression and Protein Tools

View expression patterns as electronic fluorescent pictographs or heatmaps, explore promoters, identify protein-protein interactions and more.



Molecular Markers and Mapping Tools

Perform Next Generation Mapping, or generate your own markers using our molecular marker tools.



Other Genomic Tools and Widgets

Remove duplicates, perform multi-dimensional Venn analyses, or generate random lists of identifiers.

BAR: Gene expression in crops and other plants



































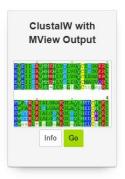


BAR: Tools and widgets

Other Genomic Tools and Widgets

Remove duplicates, perform multi-dimensional Venn analyses, or generate random lists of identifiers.



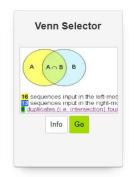


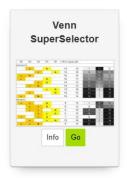














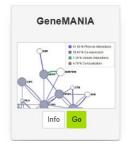


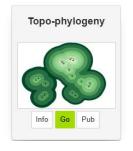










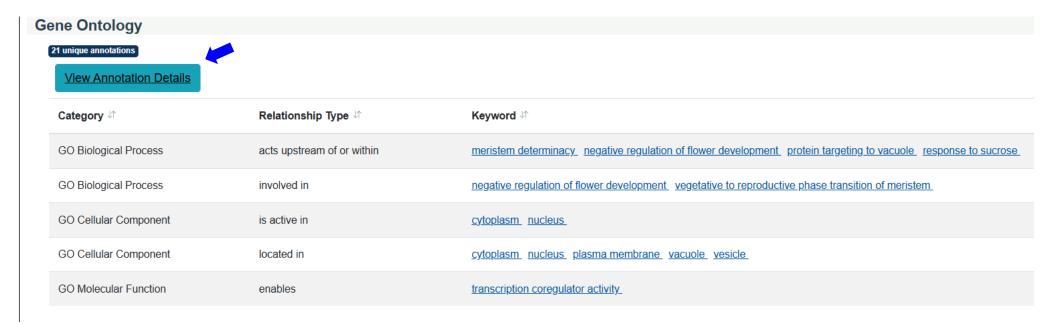






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Gene ontology (GO)



Functional annotations with **controlled vocabulary** terms:

Gene Ontology (GO)

Genes function and **localization** of the gene product.

Most gene annotations are made by curators using the <u>published literature</u> as sources. Some annotations are based on <u>computational methods</u>. Detailed information including references and supporting evidence can be obtained by clicking on the link to view Annotation Detail.

Annotation details

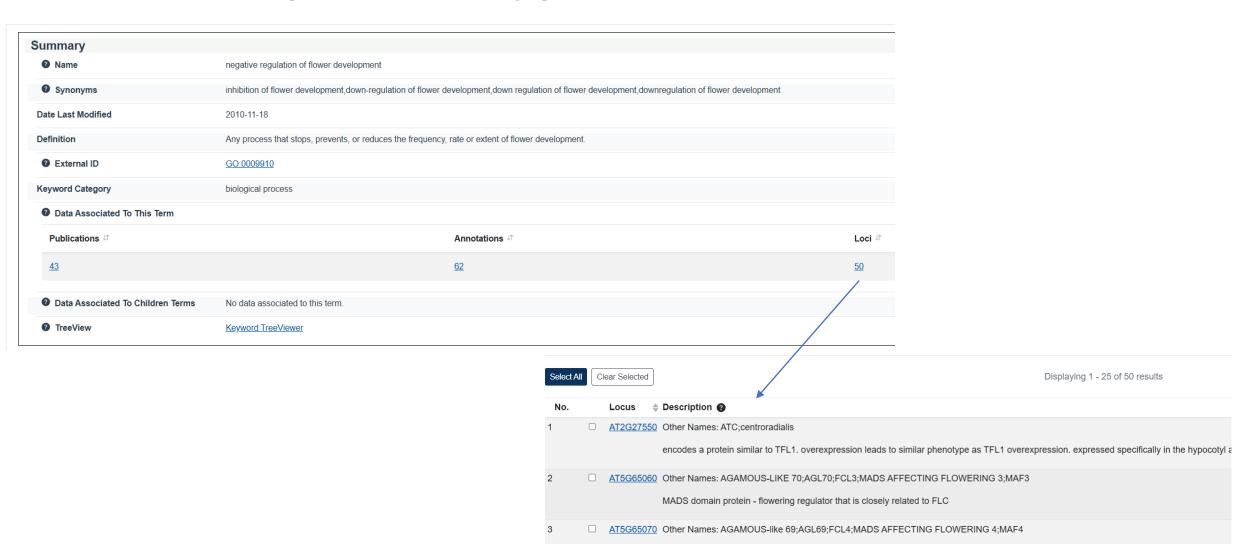
Select All Clear Selected

Displaying 1 - 23 of 23 results

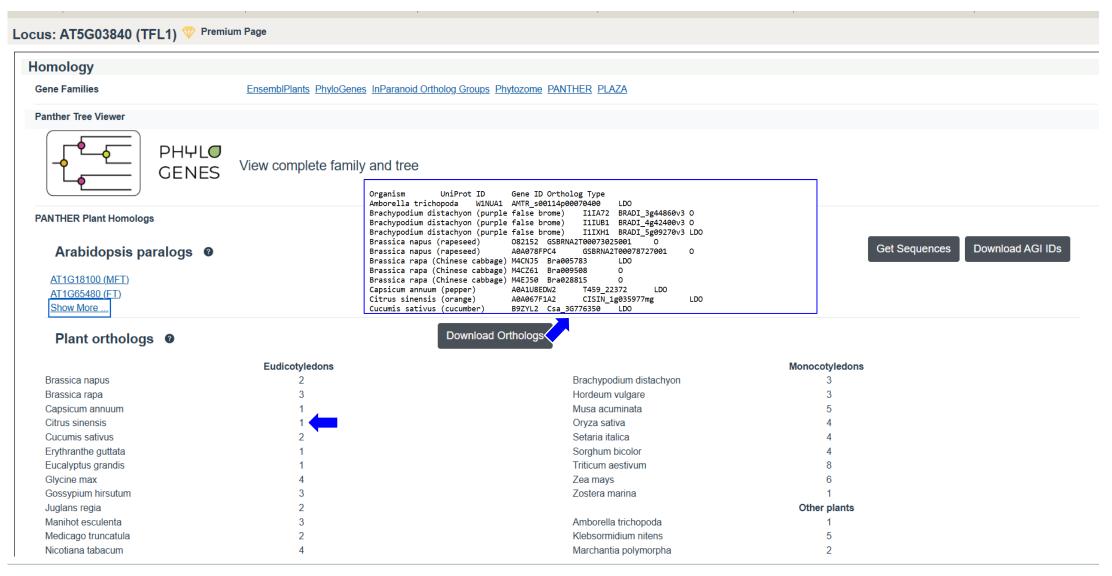
Results per page (top): 25

No.	♦ Gene Locus Model	Gene Symbol/Full Name	Relationship ♦	Keyword	Keyword ♦ Category	Evidence:Description:With	Reference
1	□ <u>AT5G03840.1</u>		located in	<u>cytoplasm</u>	cellular component	inferred from sequence model: predicted protein features:	AtSubP analysis prediction
2	□ <u>AT5G03840</u>	TFL1/TERMINAL FLOWER 1	acts upstream of or within	response to sucrose	biological process	inferred from mutant phenotype: mutant growth experiment with supplementation of substrates: NONE	Ohto et al (2001)
3	□ <u>AT5G03840</u>	TFL1/TERMINAL FLOWER 1	acts upstream of or within	negative regulation of flower development	biological process	inferred from mutant phenotype: analysis of physiological response: NONE	<u>Shannon et al</u> (1991)
4	□ <u>AT5G03840</u>	TFL1/TERMINAL FLOWER 1	acts upstream of or within	protein targeting to vacuole	biological process	inferred from mutant phenotype: biochemical/chemical analysis: NONE	Sohn et al (2007)
5	□ <u>AT5G03840</u>	TFL1/TERMINAL FLOWER 1	involved in	vegetative to reproductive phase transition of meristem	biological process	Inferred from Biological aspect of Ancestor: none: PANTHER:PTN002260847 TAIR:lo cus:2038643	Annotation inferences using phylogenetic trees prediction

Using GO to identify genes with similar functions



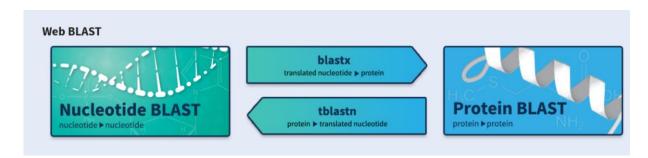
Finding crop orthologs



Finding orthologs with Blast search



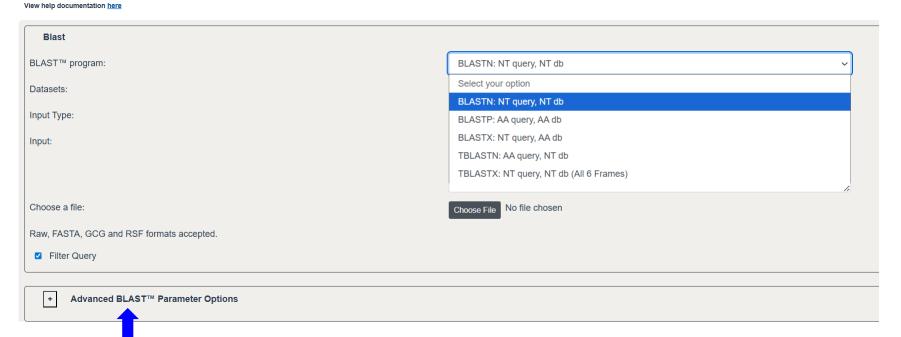
NCBI https://blast.ncbi.nlm.nih.gov/Blast.cgi



- search homologs
- map transcripts
- examine the specificity of primers

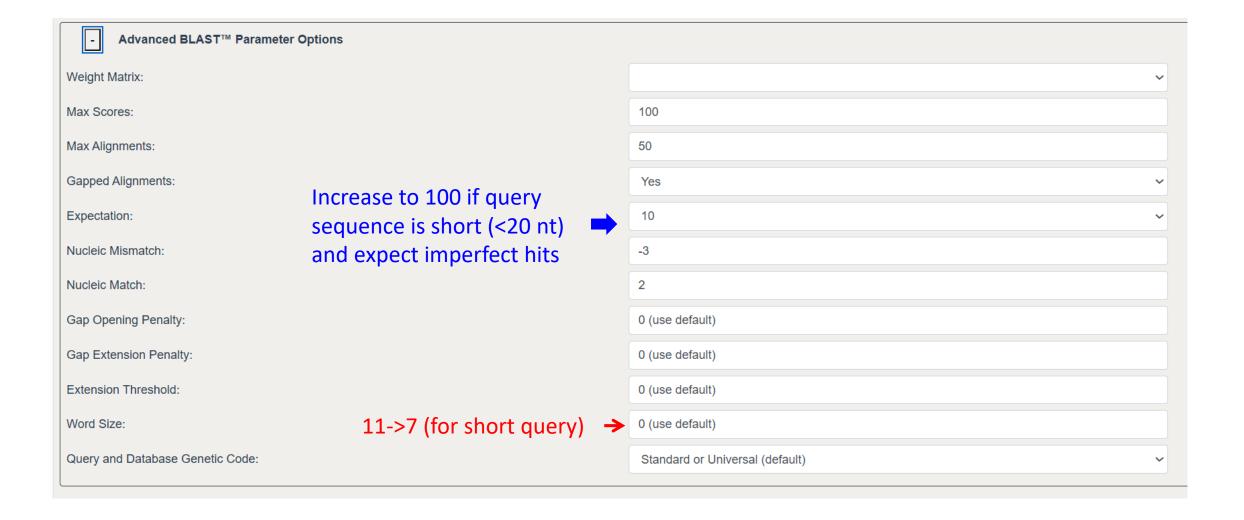
Blast: homolog search

Protein search is more sensitive than nucleotide search Use BLASTP or BLASTX for searching homologs



TAIR BLAST 2.9.0+
This form uses NCBI BLAST 2.9.0+

Blast search: short sequences (e.g. primers)



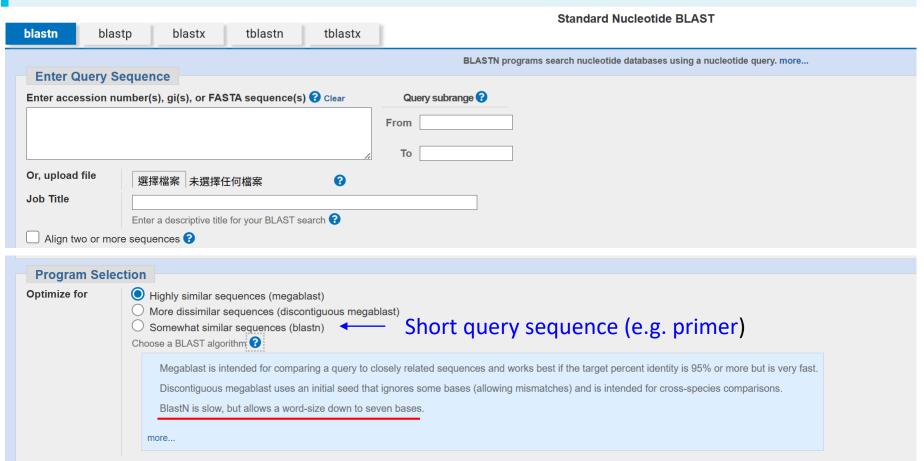


BLAST® » blastn suite Home Recent Res

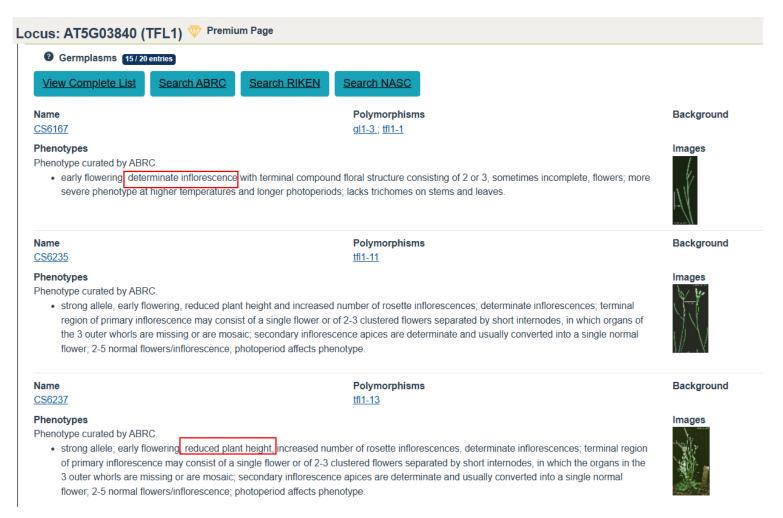


Important update

The ClusteredNR database is now the default Protein BLAST database. Learn more about ClusteredNR



Mutants and phenotypes

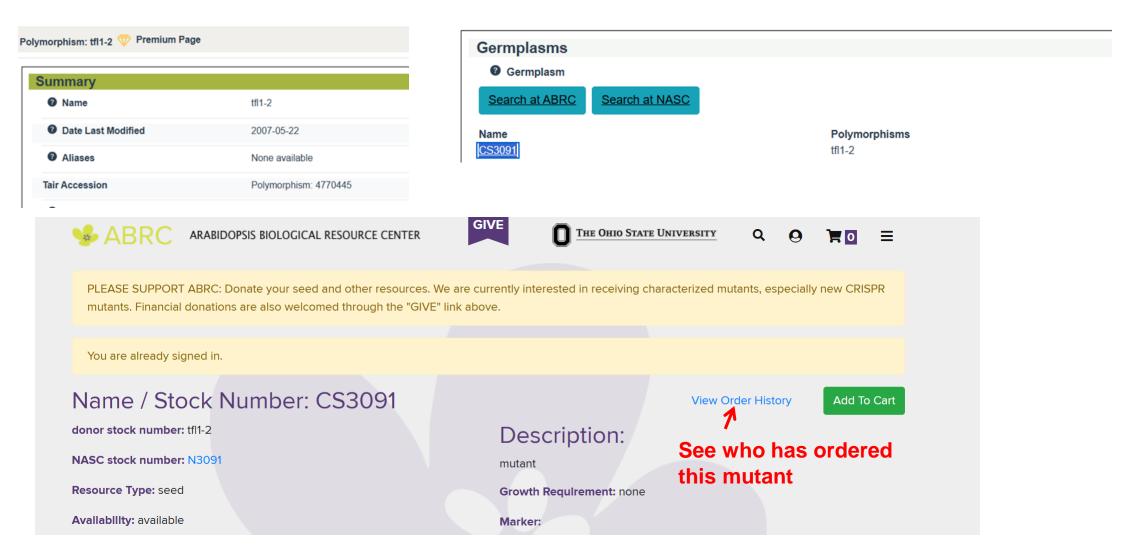




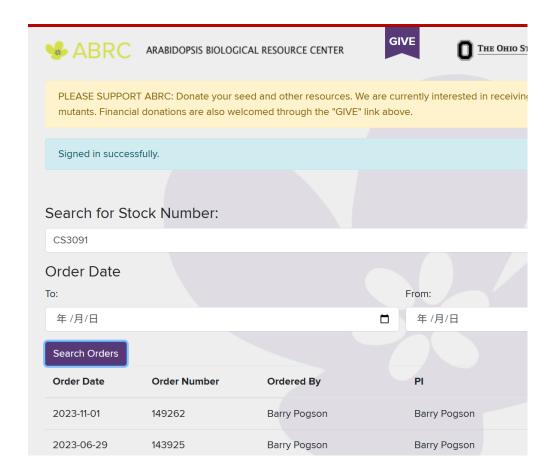


Mutant order through ABRC (not our ABRC)

Need to apply an account for making order



Stock order histories



2013-11-05	T104218	Chung-Mo Park	Chung-Mo Park
2013-08-16	T102573	Ariel Orellana	Ariel Orellana
2013-04-05	T99460	Vi Nguyen	Yuhai Cui
2013-03-21	T99143	Zheng Wang	Zhixi Tian
2012-12-19	T97305	Ying Wang	Paula Pijut
2012-11-14	T96529	Huazhong Ren	Huazhong Ren
2012-09-27	T95467	Hidetoshi Ikegami	Hidetoshi Ikegami
2012-05-27	T92681	Bin Liu	Bin Liu
2012-05-15	T92386	Tien-Shin Yu	Tien-Shin Yu
2012-04-11	T91510	Byeong-ha Lee	Byeong-ha Lee

Banana genome

- Banana: AA, AAA, BB, AAB, AAAB genome
- Triploid banana: sterile
- A reference A genome (DH Pahang): 2012 (v1); 2021 Telomere-to-telomere (v4)
- AAA Cavendish: 2023



Mapping genes in crops

Identify candidate genes responsible for a trait

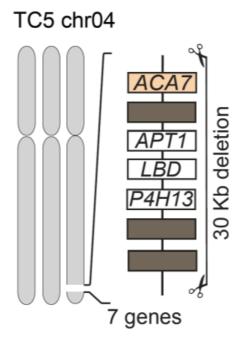
e.g. Disease resistance



mutations

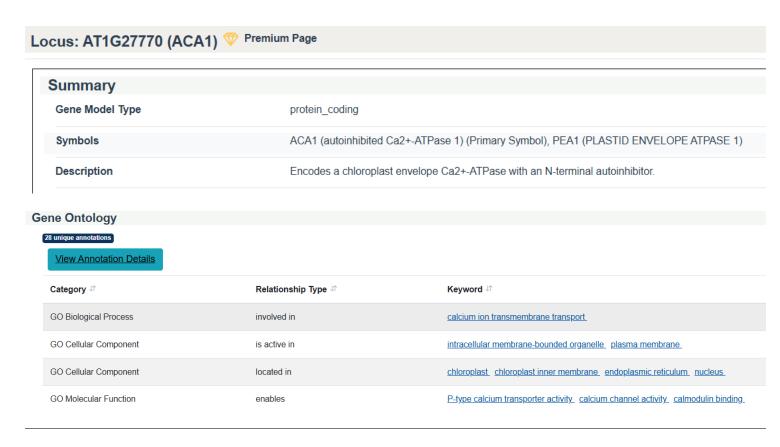
TC5

Hypothesis: A negative regulator of plant immunity



Finding Arabidopsis orthologs

- AmTrACA B AtACA1 AtACA10 L_{MaACA7}
- 1. BLAST banana protein against TAIR protein database
- 2. Identify orthologs
- 3. Check the functions Arabidopsis orthologs



Functions of Arabidopsis orthologs

- Annotations could be incomplete or incorrect
- Read key papers

blications			
15 / 15 entries			
		Results per par	age: 10 ¢
Author/Title ∜↑	Source ↓↑	Associated Loci ⊍↑	Date √1
Wang, S., Zhang, C., Chen, R. et al. H2S is involved in drought-mediated stomatal closure through PLD?1 in Arabidopsis	PLANTA	AT1G12480; AT1G27770; AT2G13610; AT2G26770; AT2G38170; AT3G15730; AT3G52720; AT3G62130; AT4G33950	2024
Kramer, M. C., Kim, H. J., Palos, K. R. et al. A Conserved Long Intergenic Non-coding RNA Containing snoRNA Sequences, IncCOBRA1, Affects Arabidopsis Germination and Development	FRONT PLANT SCI	AT1G05907; AT1G05913; AT1G05917; AT1G18080; AT1G27770; AT3G03415; AT3G03425; AT3G03445; AT3G05655	2022
Rahmati Ishka, M., Brown, E., Rosenberg, A. et al. Arabidopsis Ca2+-ATPases 1, 2, and 7 in the endoplasmic reticulum contribute to growth and pollen fitness	PLANT PHYSIOLOGY	AT1G27770; AT2G14610; AT2G41560; AT3G57260	2021
Ascencio-Ibanez, Jose Trinidad, Sozzani, Rosangela, Lee, Tae-Jin et al. Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection.	PLANT PHYSIOLOGY	AT1G01010; AT1G01030; AT1G01040; AT1G01060; AT1G01080; AT1G01090; AT1G01120; AT1G01140; AT1G01190; AT1G01290; AT1G01300; AT1G01320; AT1G01340; AT1G01350; AT1G01430; AT1G01470; AT1G01550; AT1G01560; AT1G01620; AT1G01725; more	2008
Mahé, Laetitia, Combes, Marie-Christine, Lashermes, Philippe et al. Comparison between a coffee single copy chromosomal region and Arabidopsis duplicated counterparts evidenced high level synteny between the coffee genome and the ancestral Arabidopsis genome.	PLANT MOLECULAR BIOLOGY	AT1G01110; AT1G01140; AT1G03800; AT1G04080; AT1G04150; AT1G04160; AT1G04190; AT1G04210; AT1G04220; AT1G04440; AT1G05460; AT1G08840; AT1G16800; AT1G19440; AT1G22610; AT1G25450; AT1G27770; AT1G30270; AT1G34320; AT1G51570; more	2007
Schmidt, Ulrike G, Endler, Anne, Schelbert, Silvia et al. Novel Tonoplast Transporters Identified using a Proteomic Approach with Vacuoles Isolated from Cauliflower Buds.	PLANT PHYSIOLOGY	AT1G01790; AT1G04630; AT1G04750; AT1G06470; AT1G08480; AT1G09330; AT1G10950; AT1G16390; AT1G16780; AT1G17840; AT1G19910; AT1G26310; AT1G27770; AT1G29310; AT1G32210; AT1G32400; AT1G34610; AT1G42960; AT1G53210; AT1G53840; more	2007

Functions of Arabidopsis orthologs



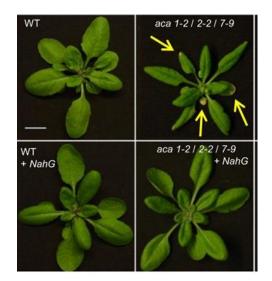


Volume 185, Issue 4

JOURNAL ARTICLE

Arabidopsis Ca²⁺-ATPases 1, 2, and 7 in the endoplasmic reticulum contribute to growth and pollen fitness ⁶⁸⁹

Maryam Rahmati Ishka ™, Elizabeth Brown, Alexa Rosenberg, Shawn Romanowsky, James A Davis, Won-Gyu Choi, Jeffrey F Harper ™ Author Notes

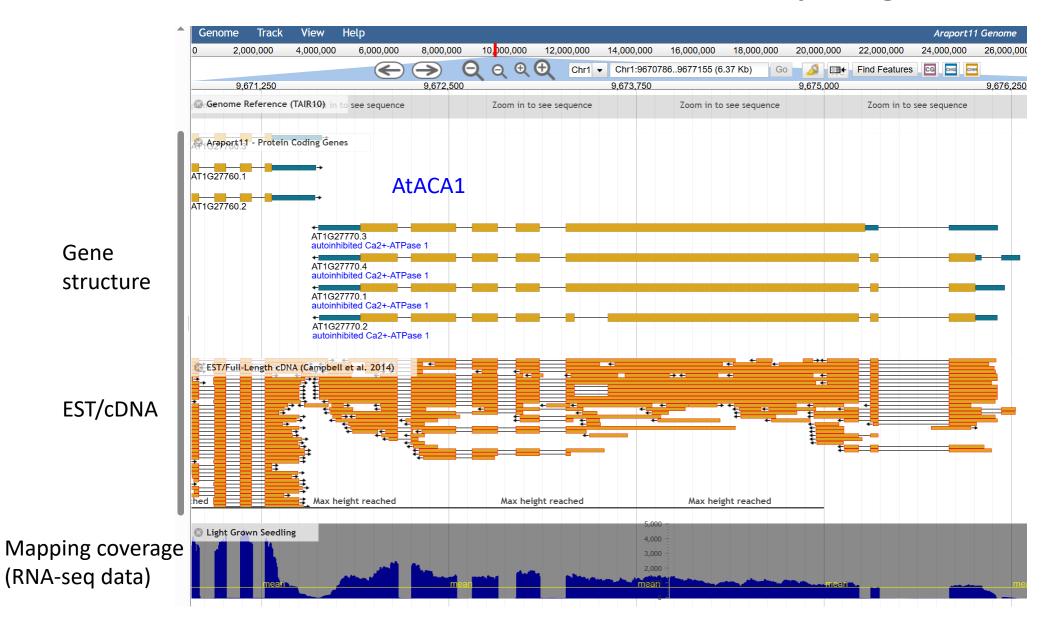


SA-dependent rosette size reductions and leaf lesions

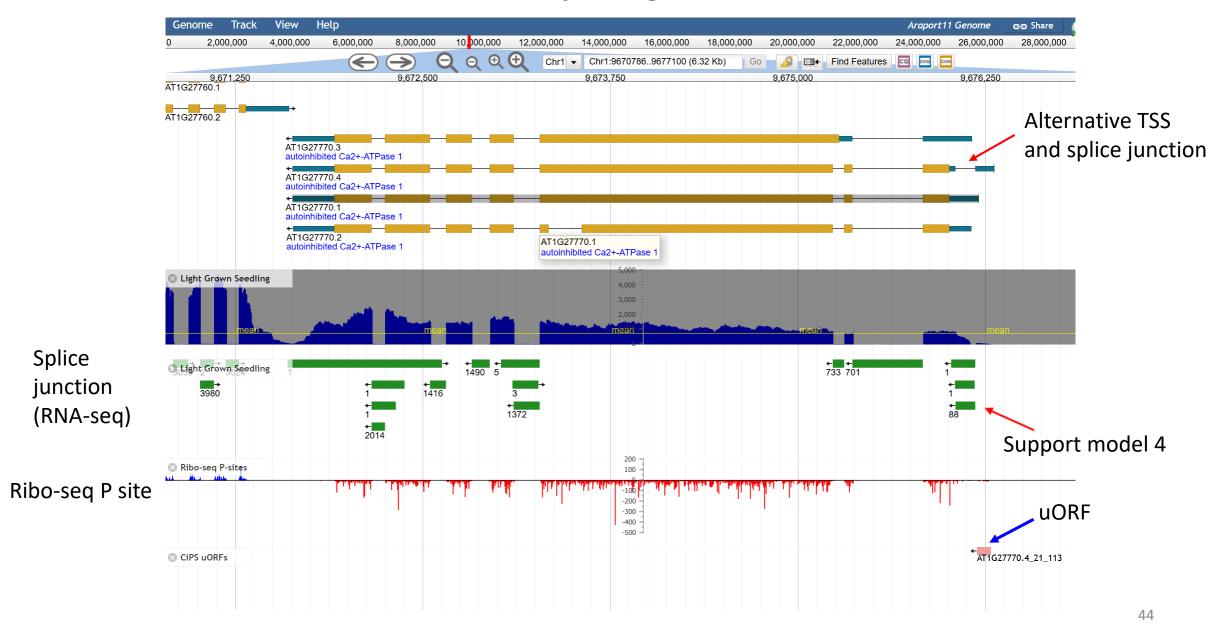
Abstract

Generating cellular Ca²⁺ signals requires coordinated transport activities from both Ca²⁺ influx and efflux pathways. In Arabidopsis (Arabidopsis thaliana), multiple efflux pathways exist, some of which involve Ca²⁺-pumps belonging to the Autoinhibited Ca²⁺-ATPase (ACA) family. Here, we show that ACA1, 2, and 7 localize to the endoplasmic reticulum (ER) and are important for plant growth and pollen fertility. While phenotypes for plants harboring single-gene knockouts (KOs) were weak or undetected, a triple KO of aca1/2/7 displayed a 2.6-fold decrease in pollen transmission efficiency, whereas inheritance through female gametes was normal. The triple KO also resulted in smaller rosettes showing a high frequency of lesions. Both vegetative and reproductive phenotypes were rescued by transgenes encoding either ACA1, 2, or 7, suggesting that all three isoforms are biochemically redundant. Lesions were suppressed by expression of a transgene encoding NahG, an enzyme that degrades salicylic acid (SA). Triple KO mutants showed elevated mRNA expression for two SA-inducible marker genes, Pathogenesis-related1 (PR1) and PR2. The aca1/2/7 lesion phenotype was similar but less severe than SA-dependent lesions associated with a double KO of vacuolar pumps aca4 and 11. Imaging of Ca²⁺ dynamics triggered by blue light or the pathogen elicitor flg22 revealed that aca1/2/7 mutants display Ca²⁺ transients with increased magnitudes and durations. Together, these results indicate that ERlocalized ACAs play important roles in regulating Ca²⁺ signals, and that the loss of these pumps results in male fertility and vegetative growth deficiencies.

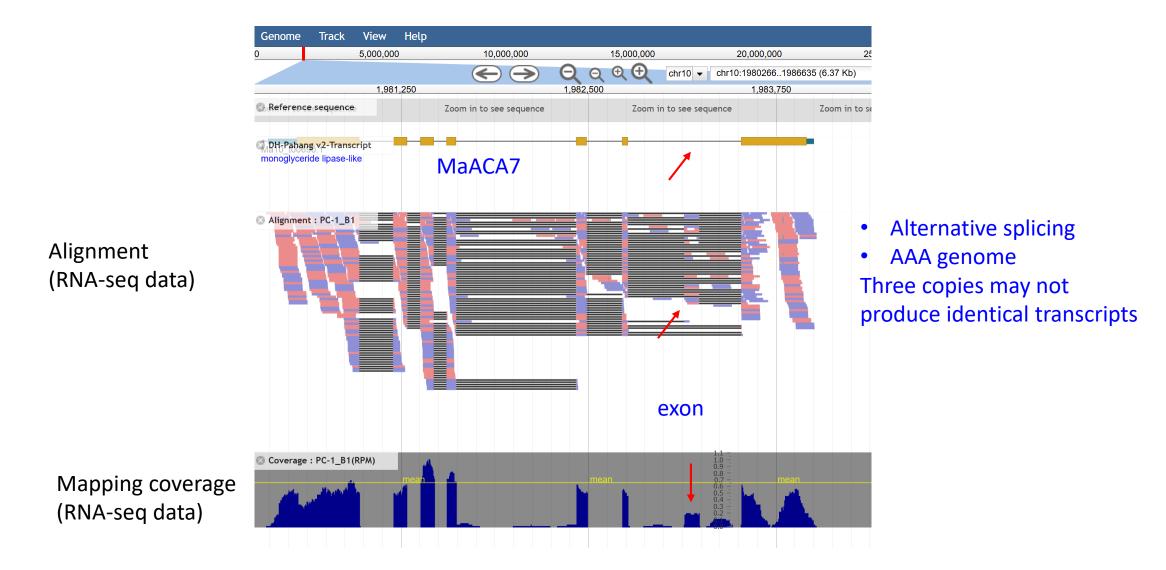
Gene structure and alternative splicing



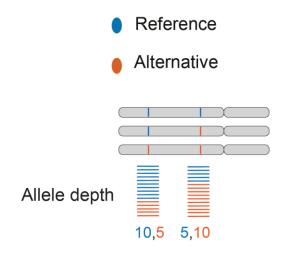
Alternative splicing and uORFs



In-house Jbrowse of banana: gene model

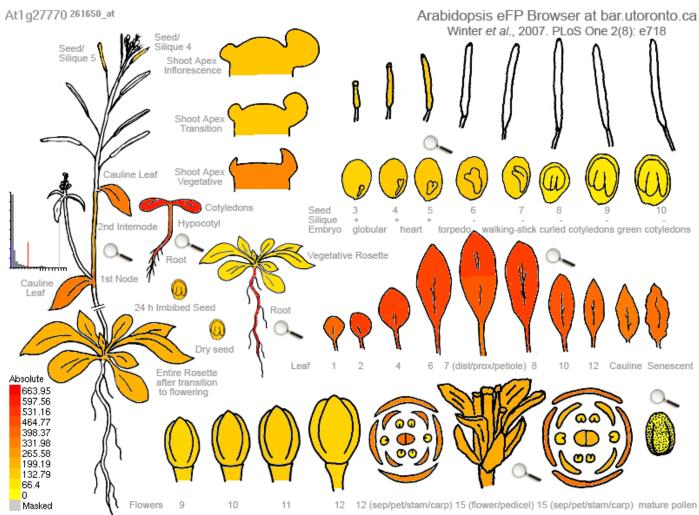


In-house Jbrowse of banana: sequence polymorphism





Locus page: Development map expression

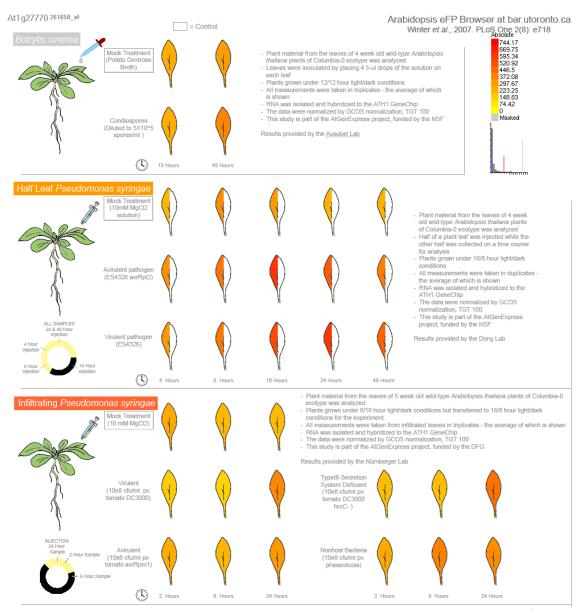


eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

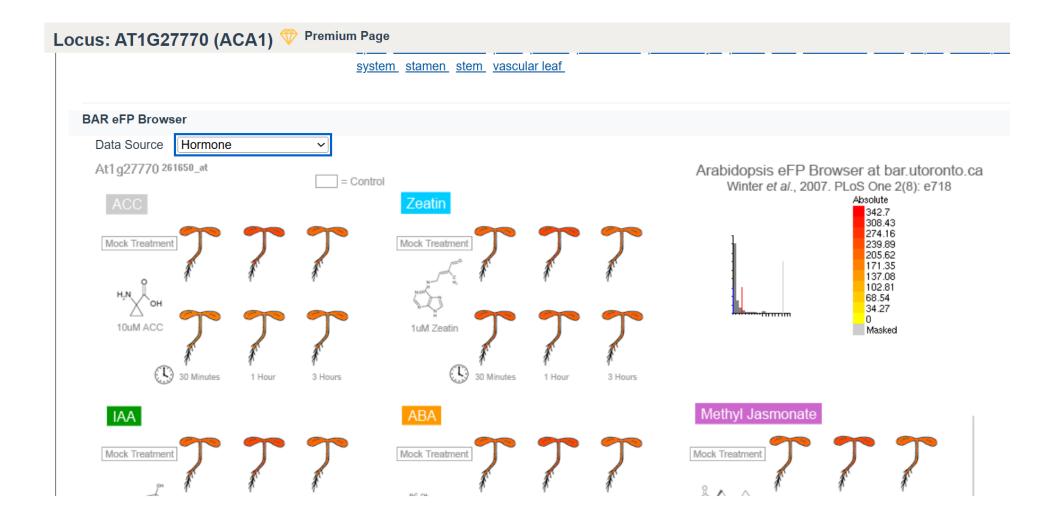
Abiotic and biotic stresses



eFP Browser Stress Series by B. Vinegar and D. Winter, drawn by D. Winter. Data from AtGenExpress Abiotic Stress Series fr



Hormone response



3 suggestions for next-generation researchers

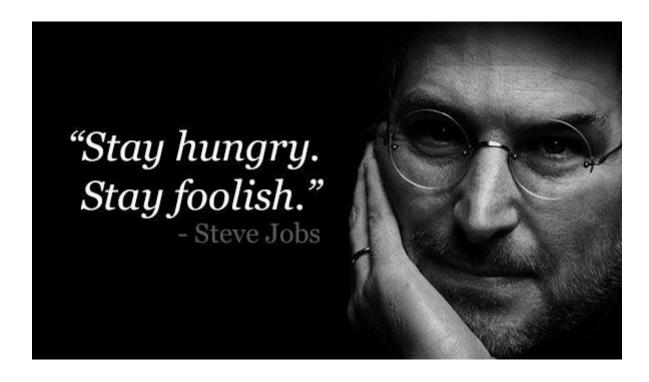
Play like a kindergarten child (no need to read the whole manual)

Warning: For molecular biology experiments, DO read the whole protocol carefully



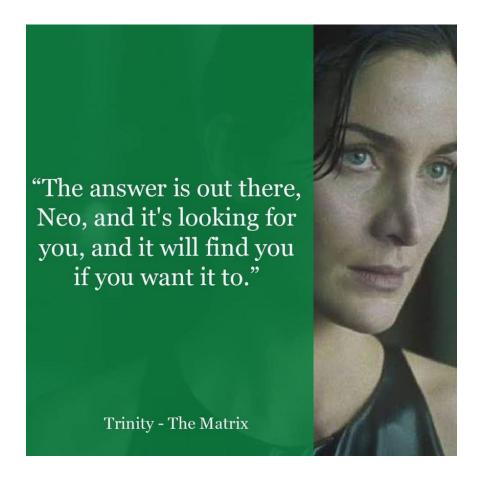
https://tw.123rf.com/

Suggestions for next-generation researchers



(ask as many questions as possible; it is free)

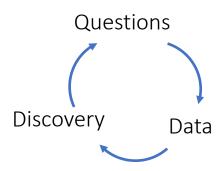
Suggestions for next-generation researchers



https://www.facebook.com/thematrixquotes/

Questions for TAIR

- To what extent is known for the gene of your interest?
 - DNA, RNA, protein sequences, isoforms
 - Functions and cellular localization (predicted, supported by experimental evidence)
 - Gene expression (spatial, temporal, response to stress, hormone ...)
 - Others: mutants, small RNAs, RNA degradation, ribosome footprints and DNA methylation
- Are there other genes having similar features as the gene of your interest?
 - Similar DNA, RNA, protein sequences
 - Similar functions
 - Similar expression pattern
 -

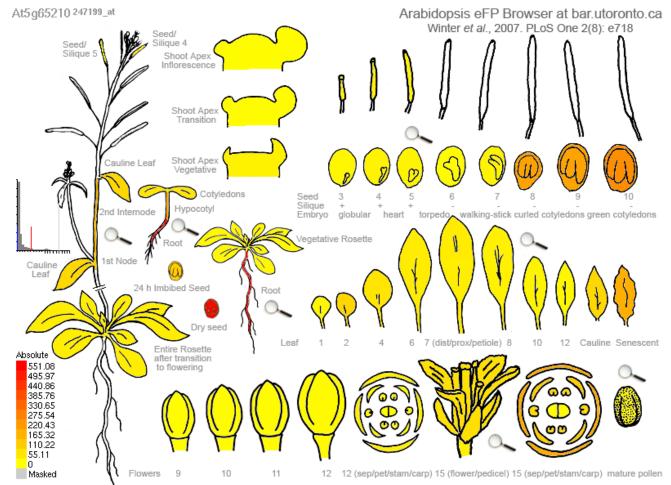




Exercise II

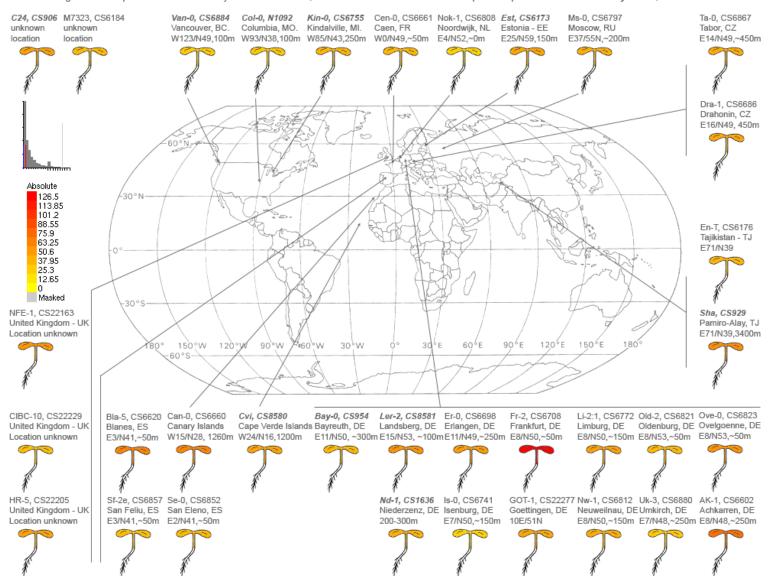
TGA1 (AT5G65210)

- 1. GO biological process: number of keywords?; number of loci in each keyword?
- 2. In annotation, what functions are supported by experimental results?
- 3. RNA expression: highest in which tissue, at what stage, in which ecotype? diurnal gene? response to any stress or hormone?



eFP Browser by B. Vinegar, drawn by J. Alls and N. Provart. Data from Gene Expression Map of Arabidopsis Development: Schmid et al., 2005, Nat. Gen. 37:501, and the Nambara lab for the imbibed and dry seed stages. Data are normalized by the GCOS method, TGT value of 100. Most tissues were sampled in triplicate.

Natural Variation eFP Browser. Data from the Weigel Lab (Lempe et al., 2005, PLoS Genetics 1:e6). Aerial parts of 4 day old seedlings greenhouse-grown in soil at 23C under continuous light were sampled. Data normalized by the GCOS method, TGT value of 100. Plant material sampled in triplicate where indicated by italics, otherwise once.





Thank you!

For questions, you can contact

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